Claim 4, (as found in the Amendment of July 30, 1985), line 10, (subparagraph b) between "acceptable" and "carrier" insert -- inert --.

REMARKS

Reconsideration of the claims of record is requested in view of the above amendments and following remarks. Applicants' acknowledge with appreciation the indication that claims 1-3, 5-8, and 13-16 contain allowable subject matter.

Claims 1-20 are pending in the application.

In the Office Action claims 2-12, 14, 15, and 17-20 were rejected under 35 USC 112, first and second paragraphs. In particular, the nomenclature of claims 2, 3, 7, 8, 10, 11, 14, 15, 18 and 19 was requested to be corrected. While the original nomenclature presented in these claims is correct, applicants have amended the claims to include more particularly the position of the lactone (i.e., hexadecadienoic-1,3-acid) to expedite prosecution.

As suggested in the Office Action, claim 4 has been amended to recite an "inert" carrier material. This renders its rejection moot.

Based upon the above, withdrawal of the rejection of claims 2-12, 14, 15 and 17-20 under 35 U.S.C. 112, first and second paragraphs is solicited.

In the Office Action the specification was objected to and claims 9-12 and 17-20 were rejected under 35 U.S.C. 112, first paragraph as allegedly failing to provide an enabling disclosure for the use of compound I in the prevention or treatment of obesity in mammals. According to the Office Action, inhibition of pancrease lipase by compound I only increases the excretion of triglycerides. "Weight loss has not, to the Examiner's knowledge, been directly correlated with decreased lipid levels absent other factors...." Applicants respectfully traverse this rejection.

Applicants reaffirm the arguments presented in the Office Action of July 30, 1985. In view of the strong inhibition action of compound I on pancrease lipase, fats (i.e., triglycerides) are excreted in unchanged form so that the amount of fat absorbed by the body fat cells is reduced, and compound I thus is effective in preventing or treating obesity in mammals. Applicants respectfully submit that except for perhaps mere speculation, there is nothing of record to doubt the truth or accuracy of any statement in this specification concerning the anti-obesity activities of applicants' claimed compounds. See In re Marzocchi, 169 U.S.P.Q. 367, 370 (CCPA 1971).

Moreover, applicants' submit the following publications which set forth experimental data for another pancrease lipase inhibitor which shows a correlation between decreased lipid levels and weight loss.

1. Comai et al., "Anti-Obesity Activity of Pluronic L-101", International Journal of Obesity 4:31-42 (1980);

2. Puls et al., "Inhibitors of the Rate of Carbohydrate and Lipid Absorption by the Intestine", Novel Approaches and Drugs for Obesity (Ed. Sullivan et al., John Libbey & Company Ltd., London 1985) pages 181-190, particularly 185-186.

According to Comai et al., agents which decrease the absorption of dietary lipids have been considered as an approach for therapy of obesity. Applicants suggest that the mechanism of action of pancrease lipase inhibitors is to prevent absorption of dietary fat, thus decreasing the lipid available for storage in adipose tissue. In the article pluronic L-101, a potent inhibitor of pancrease lipase, was examined and its obesity potential was evaluated in vivo in rats. As reported in the article, pluronic L-101 produced a significant and dose-dependent decrease in body-weight gain while not effecting food consumption. This article, thus, correlates inhibition of pancrease lipase (Fig. 1), fat absorption (Table I) and weight loss (Table II).

Furthermore, pancrease lipase inhibitors such as compound I inhibit the splitting of dietary fat. This has been simulated experimentally by substituting normal dietary fat for artificial fat (e.g. sucrose polyester) which is resistant to splitting from pancrease lipase. In these experiments, the subject reacted as expected and exhibited weight loss and

reduction of plasma total and LDL cholesterol. Two articles which describe the above are as follows:

1) Glueck, et al., "Sucrose Polyester: Substitution for Dietary Fats in Hypocaloric Diets in the Treatment of Familial Hypercholesterolemia", Am. J. Clin. Nutr. 37, 347-354, (1983)

2) Jandacek, R.: "Studies with Sucrose Polyester," Novel Approaches and Drugs For Obesity, (Ed. Sullivan et al. 1985, John Libbey & Company Limited, London, 1985,) pp. 13-21.

Accordingly, these articles again show a correlation between lipid availability and weight loss.

From the above, applicants' submit that the specification and claims 9-12 and 17-20 satisfy the requirements of 35 U.S.C. 112, first paragraph.

Allowance of the claims of record are solicited.

The Examiner is hereby authorized to call the undersigned attorney of record "collect" on any matter connected with this application. The telephone number is Area Code (201) 235-3656.

In the absence of the undersigned attorney of record, the call will be accepted by another attorney empowered in this application.

Respectfully submitted,

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Novel approaches and drugs for obesity

Proceedings of a Satellite Symposium to the Fourth International Congress on Obesity, held in New York, USA, 3-5 October 1983

Edited by
Ann C. Sullivan *USA*
Silvio Garattini *Italy*

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STUDIES WITH SUCROSE POLYESTER

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Sucrose polyester (SPE) is a lipid synthesized from sucrose and fatty acid methyl esters. SPE has physical and organoleptic properties that closely resemble triglycerides, but it is not hydrolysed in the intestine and therefore not absorbed. By providing a persistent lipophilic phase in the intestine, SPE reduces the absorption of lipophilic substances such as cholesterol. SPE leaves the stomach more rapidly than triglyceride oils, presumably because of the absence of an effect on duodenal receptors. In studies with obese subjects, dietary SPE reduced total and LDL cholesterol by reducing the absorption of cholesterol from the intestine. In a study, in which SPE was covertly substituted for dietary fat, ten obese subjects in a weight-loss regimen did not increase dietary intake to compensate for the energy removed from the diet by SPE substitution. In a similar study with five naive, obese subjects, the investigators concluded that the mean energy intake decrease during SPE treatment [184 (769 kJ) kcal/d] was not significant. SPE continues to be studied as a means of reducing energy intake.

Introduction

Sucrose polyester (SPE) is a lipid with physical and organoleptic properties that are virtually identical to those of triglycerides. It differs from triglycerides in one important and unique property in that it remains inert in the presence of the fat-splitting digestive enzymes of the pancreas. This lack of biochemical activity has initiated studies that have sought to understand and explore the potential of this nonhydrolysable, nonabsorbable lipid. I will review the history, properties, and effects of SPE, and then focus on some applications of SPE as a tool to understand, and possibly treat, obesity and its complications.

The resistance of SPE to pancreatic lipases was discovered by Mattson and Volpenhein[15], who were measuring the rates of lipolysis for a series of polyol oleate compounds. As they increased the number of ester groups per molecule they found a maximum lipolysis rate for three ester groups (triolein), a lower rate for four (erythritol tetraoleate), and the absence of reaction for six (sorbitol hexaoleate), and eight (sucrose octaoleate). The relative rates appear as a bar graph in Fig. 1. Their subsequent studies showed the absorption of these esters to be dependent on the extent of their hydrolysis[16]. Absorption decreased with the increasing number of ester groups and was lower than measurable limits for sorbitol and sucrose oleate esters[17]. These reports provided convincing data that supported the concept that hydrolysis of fat is a prerequisite to its absorption. The validity of this mechnism, which requires hydrolysis prior to absorption of fats, has been provided by the methodology of physical chemical[11] and electron microscopic studies[3].

The synthesis and isolation of relatively large quantities of highly-esterified sucrose result in a mixture of the hexa-, hepta- and octaesters[24], which we have termed sucrose polyester, or SPE (Fig. 2). Like sucrose octaoleate, this mixture of esters is inert in the presence of lipase and, therefore, not absorbed[7].

Properties of SPE

The physical properties of SPE are determined by the fatty acid constituents of the compounds[12]. SPE prepared from high-melting fats is itself a high-melting fat. SPE prepared from oils such as safflower oil or soybean oil is a liquid oil that resembles the source oil.

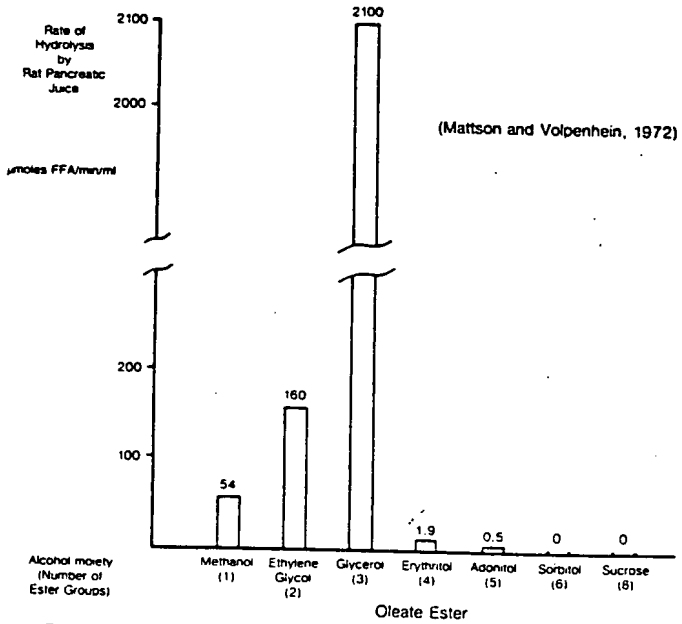
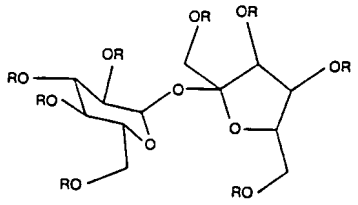


Fig. 1. *A comparison of the rates of hydrolysis of polyol esters*[15]



Sucrose Polyester (SPE)

R>6 CH_3-$(CH_2)_n$-C-OH

R<2H

Fig. 2. *Sucrose polyester (SPE), the hexa-, hepta- and octa-long chain fatty acid esters of sucrose*

The interfacial behavior, taste, appearance, density and aroma are essentially the same as those of triglycerides with the same fatty acid composition. It can be incorporated into most foods as a replacement for triglycerides without changing the taste and texture.

The most succinct description of the biochemistry of SPE is that it is inert. Such a statement may be misleading, however, since a nonhydrolysable and nonabsorbable fat in the intestine can have indirect biochemical consequences by changing the normal absorption of biochemically active substances such as cholesterol or fat-soluble vitamins. This 'solvent effect' will be briefly summarized in the following section on lipophile absorption in the presence of SPE.

Effects of SPE on absorption and resorption

For many lipophilic materials, such as cholesterol and vitamin A, the absorption from the intestine is apparently dependent on dissolution of the material in the aqueous phase of

the intestine. The dispersion into the aqueous system is provided by dissolution into small micellar aggregates of bile salts and monoglycerides.

After triglyceride fat is ingested, it is hydrolysed into monoglycerides and fatty acids which are rapidly absorbed. The oil phase disappears quickly by absorption in the jejunum. A nonabsorbable lipid, such as SPE, disappears only by intestinal transit so that a persistent oil (organic) phase is present in the intestine during the absorption of other lipophilic materials. This oil phase competes with the micelles for lipophilic molecules with a resulting hindrance of absorption for many such compounds. This mechanism is summarized in Fig. 3. When half of the triglyceride was replaced by SPE in the diet of rats, significant reductions in absorption of lipophilic compounds resulted (Table 1). The absorption of cholesterol[18], vitamin A[19] and DDT[28] was reduced by SPE, while the absorption of chenodeoxycholic acid[13], a relatively hydrophilic material, was not affected.

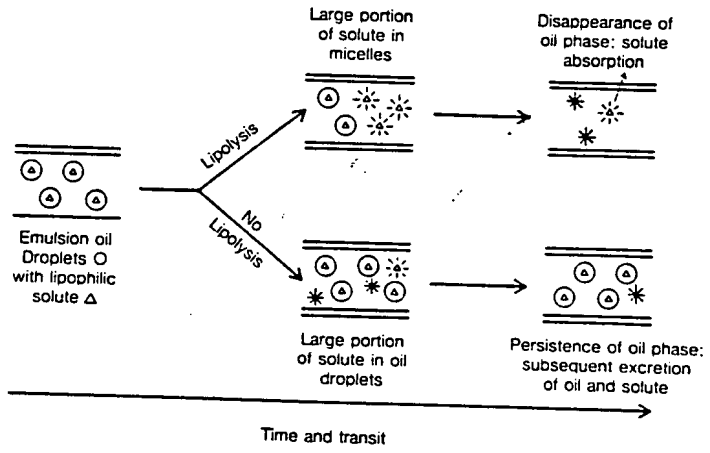


Fig. 3. *The effects of a nonabsorbable lipid on the absorption of lipophiles*

SPE can also affect lipophilic compounds that enter the small intestine in bile or by intestinal secretion. Dissolution into the SPE phase, intestinal transit and fecal excretion reduce the intestinal reabsorption of these compounds. Cholesterol in bile is poorly resorbed and excreted in feces in the presence of SPE[13]. A novel application of this effect is in the removal of lipophilic toxins that persist in adipose tissue. Mutter *et al.* recently reported that the rate of fecal excretion of DDT and its metabolites from gerbils was increased up to 10-fold when SPE was administered in combination with a low energy diet[20]. Presumably the energy reduction mobilized the deposited DDT so that it could be excreted by biliary and nonbiliary means into the intestine. Its resorption from the intestine was blocked by SPE.

Work in the area of toxic lipophile removal continues in an effort to understand and utilize this potentially beneficial effect of SPE. We also continue to study the effects of SPE on vitamin absorption with focus on the supplementation of the vitamins in the SPE vehicle.

Table 1. *The reduction in the absorption of lipophilic compounds in rats when SPE replaced 50 per cent of dietary triglycerides*[18,19,13,28]

Compound	*Assay*	*Percent reduction*
cholesterol	diet-fecal balance	67
vitamin A	liver assay	42
DDT	thoracic duct cannulation	68
chenodeoxycholic acid	bile duct cannulation	0

SPE does not have an effect on the absorption of triglycerides, based on recovery of non-SPE lipids in feces[7]. There are several probable explanations for this observation. First, the triglyceride digestion products are too hydrophilic for significant dissolution into SPE. Monoglycerides prefer the micellar phase, and as the pH of the intestine increases, dissociated fatty acids may also shift to the micellar phase. Compared to many lipophiles, the rate of absorption of fatty acids and monoglycerides is very fast, and this rapid absorption may also reduce the effect of SPE. Thus the movement of fatty acid from micelle to mucosa is fast enough to keep the fatty acid flux in the SPE to micelle direction rather than the reverse. The net effect is that SPE does not reduce the absorption of the triglycerides.

As would be expected from the hydrophilic nature of the digestion products of carbohydrates and proteins, SPE does not affect the absorption of these macronutrients. Neither carbohydrate nor protein absorption is altered by the presence of dietary SPE.

The effects of SPE in the colon differ from those of triglycerides. Again the key to the behavior of SPE is its resistance to hydrolysis. The SPE that is recovered in the feces is not hydrolysed to produce free fatty acids. Triglycerides that reach the colon because of pancreatic lipase deficiency interact with enzymes of microflora to produce hydrolytic products and other metabolites. Although dietary SPE produces stools of high fat content, the uncomfortable symptoms of steatorrhea that are caused by the more hydrophilic fat digestion products do not appear.

SPE in the colon apparently sequesters some lipophiles from the microflora. In a group of subjects, who normally converted fecal cholesterol to coprostanol and coprostanone, SPE significantly reduced the extent of this degradation[14]. SPE has been reported to have little or no effect on the quantity or composition of fecal bile acids[5,8,13].

SPE and stomach emptying

SPE has been used as a tool to study the rate of gastric emptying in human volunteers. Measuring the duodenal recovery of lipophilic and aqueous markers, Cortot *et al.* found that SPE was removed from the stomach more rapidly than triglyceride fat[4]. They concluded that the ability of a fat to be hydrolysed is one of two requirements for the reduction of the rate of stomach emptying. The second requirement was that the fat must physically separate from the aqueous phase in the stomach.

Their observations are, again, consistent with the inability of SPE to be hydrolysed. Duodenal receptors that influence gastric emptying apparently respond only to the products of hydrolysis, not to intact triglycerides or to SPE.

SPE in satiety and weight loss

An obvious therapeutic application of SPE is its use as a replacement for dietary fats to reduce the intake of absorbable energy. Such a reduction in energy intake should produce an energy deficit and weight loss.

The regulation of food and energy intake, however, is extremely complex, and the simple replacement of SPE for dietary fat is not necessarily a means of producing a low energy diet. We have observed, many times, in the rat that the diet intake is energy regulated. Rats consuming a diet containing SPE *ad libitum* regulate intake by the amount of absorbable energy they eat rather than by diet mass. In other words if we add 10 per cent SPE to a baseline diet the rats begin to eat 10 per cent more diet per day compared to the baseline diet.

If regulation of stomach emptying is the determinant of satiety, Cortot's study of stomach emptying[4] suggests that SPE will not provide the satiety provided by normal dietary fat since SPE does not provide the free fatty acids to intestinal receptors.

Satiety in man, however, is quite complex and related to many factors including psychological and social ones that presumably differ from the rat, as well as the physiological ones that may or may not resemble those of the rat. In contrast to studies with the rat, a reduction

in energy density of the diet has resulted in decreased food consumption in man in studies in which the diet composition shifted toward high-carbohydrate, low-fat diets[23].

Clinical studies with SPE

SPE potentially can have two primary therapeutic benefits directly related to its non-absorbability. First, its effect on the absorption of dietary and enterohepatic cholesterol can be reflected in changes in plasma cholesterol. Second, the use of SPE as a replacement for energy-dense dietary fat can have an effect on diet composition and digestible energy intake. To examine these possibilities, four clinical studies have focused on SPE in overweight and overweight-hypercholesterolemic patients. These studies have measured parameters of cholesterol metabolism and satiety.

Cholesterol metabolism in overweight subjects

The first study of SPE and cholesterol metabolism in overweight subjects was directed by Grouse and Grundy[5]. Eleven overweight normocholesterolemic subjects were studied in a metabolic ward for 6 weeks, control period, followed by 6 weeks of treatment with 50 g/d of SPE in a liquid-formula diet. During the entire 12 weeks the subjects received 1000 (4180 kJ) kcal/d.

Total cholesterol levels decreased by 20 percent during the control period and by an additional 6.8 percent during the SPE period. Weight loss was the same for both periods. Cholesterol absorption decreased and excretion increased during the SPE treatment. Gall bladder bile saturation was not changed by SPE treatment although a trend toward reduced saturation (166±54 percent during control vs 142±26 percent) was noted during the SPE period. Low energy diets have been associated with increased biliary cholesterol saturation in obese subjects[2]. Bile acid excretion was slightly increased by SPE.

The use of SPE in overweight (20 percent above ideal) hypercholesterolemic subjects has also been reported[9]. The effects of SPE were followed in 5 obese women who were heterozygous for familial hypercholesterolemia. The study design included a 10-d basal diet period followed by a 30-d treatment period. The basal period diet was low energy, and the treatment period maintained this diet with 30 g/d of fat replaced with SPE. The SPE was incorporated as a fat substitute into foods prepared in the metabolic ward diet kitchen as well as being provided in a margarine-like bread spread.

The dietary intake was 1426±63 (5960±263 kJ) kcal/d during baseline with 40 percent of the energy as fat. Normal dietary fat decreased during SPE treatment so that absorbable dietary fat was 23 percent absorbable calories, and total caloric intake decreased to 1104±61 (4615±255 kJ) kcal/d. The polyunsaturated to saturated fat (non-SPE) ratio was 1.2 during the basal period and 1.1 during SPE treatment.

The results are summarized in Fig. 4. Significant reductions in total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) resulted. Compared to the basal period, SPE treatment reduced LDL-C by 23 percent and HDL-C by 11 percent. The changes in LDL-C and HDL-C during the basal period were 4 and 11 percent respectively. During the 10-d basal period weight decreased a mean of 1.2 kg while during the 30 day SPE treatment weight decreased a mean value of 3.7 kg. Other studies of the effects of weight loss on lipoproteins have shown decreased LDL-C[21] and both increased[6,26] and decreased[21,27] HDL-C.

This study suggests the potential benefit of SPE in the treatment of the obese hypercholesterolemic patient. The substitution for dietary fat with SPE allowed an additional reduction in energy in a low energy regimen as well as a hindrance to the absorption of dietary cholesterol. The combined effect of weight loss and SPE produced significant reductions in LDL-C.

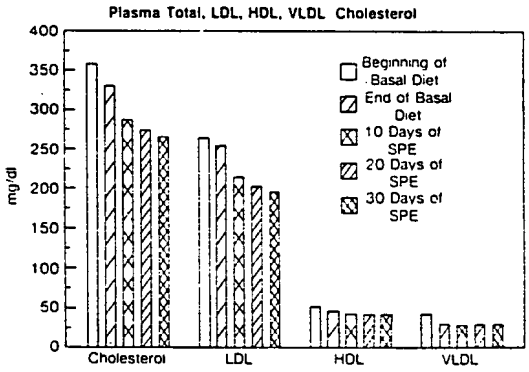


Fig. 4. *The effect of the substitution of SPE for dietary fat on plasma total and lipoprotein cholesterol* (from ref. 9, with permission)

Clinical studies of SPE and satiety

I would now like to move from the area of cholesterol metabolism to one that is equally complex, human satiety. The first examination of the possible role of SPE in human satiety was directed by Glueck at the University of Cincinnati[10]. Figure 5 summarizes the design of this study. The 10 obese subjects, who were at least 20 percent above recommended weight, entered the metabolic ward for a basal period of 7–14 d. During that period dietitians

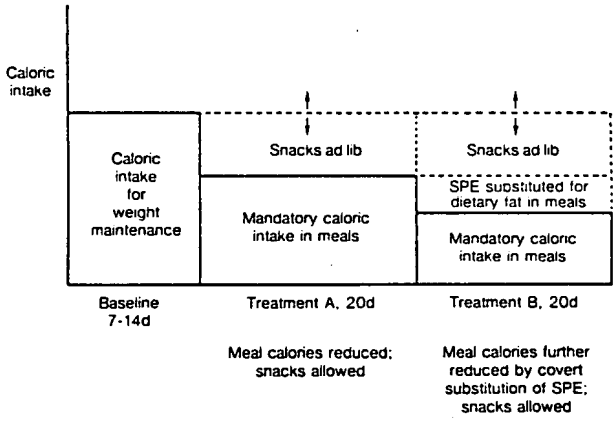


Fig. 5. *The design for a study of the covert substitution of SPE for dietary fat*[10]. Half of subjects followed sequence A,B; half B,A

determined the meal preferences of the individual and the energy intake required to maintain body weight. At the end of this basal period the subject was randomly assigned to one of two treatment sequences. In one sequence the patient entered a 20-d control period followed by a 20-d SPE period. In the other sequence this order was reversed.

The control-period diet consisted of meals that contained 60 percent of the energy ingested during the weight-maintaining basal diet period. Each subject was also given access to snacks including potato chips, bagels, fruit, soda, cookies, vegetables and pretzels. Snack energy consumption was also limited to an amount equal to the value of 60 percent of baseline meals. The SPE period was the same as the control period except that the 40 g fat/1200 (5016 kJ) kcal of meals was replaced by 40 g of SPE. Thus SPE substitution caused a further reduction of energy. Subjects were required to eat the complete meal in each of the periods.

Table 2. *The results of the covert substitution of SPE for fat in meals* (from ref. 10, with permission)

	Placebo	*SPE*
Intake from meals (kcal/d)	1801 ± 259	1270 ± 183*
snacks (kcal/d)	357 ± 99	415 ± 104
fat (g/d)	104 ± 13	54 ± 9*
plasma cholesterol		
total (mg/dl)	203 ± 16	183 ± 18*
LDL (mg/dl)	115 ± 6	99 ± 7*
HDL (mg/dl)	37.4 ± 4.1	37.5 ± 3
weight loss (kg)	3.0 ± 0.6	3.5 ± 0.7

*$P < 0.05$

The results of the study are given in Table 2. The principal variable, snack consumption, was the same for both the control and the SPE treatment periods. Thus, the subjects did not distinguish between the meals in the control and SPE periods. There was no compensation for the nonabsorbable SPE energy by an increase in snacks. Subjective questions dealing with the patients' personal hunger and satiety assessment did not distinguish between the two periods. In this study the subjects did not behave like the rats that eat on an isocaloric basis. LDL-C was lower during the SPE period, and weight loss was not significantly different from the control period.

I would like to briefly comment on one other study which has been recently completed by Porikos and coworkers at the Obesity Research Center at St Lukes (Roosevelt Hospital Center in New York). I will not discuss this study in detail since Dr Porikos outlines the study elsewhere in this supplement.

The design consisted of a two 10-d baseline periods separated by an 18-d treatment period. Five subjects were enrolled with body weight more than 20 per cent above ideal. All meals were given *ad libitum*, and subjects made no attempt to restrict dietary intake. They were not aware that satiety was being evaluated. During the treatment period SPE replaced conventional fats in the prepared diets. The resulting energy dilution was 10 per cent, and, on the average, SPE replaced 41 g or 370 (1547 kJ) kcal of fat per d.

Although the mean energy intake during SPE treatment was 184 (769 kJ) kcal/d less than during the control, this was not statistically significant. The investigators concluded that the subjects compensated for the fat replacement. There was, however, individual variability among subjects in the study since two showed apparent decreases in energy intake during SPE treatment. There was a change in dietary composition as the percent of absorbable energy as fat decreased during the treatment period.

The difference in the results of the Glueck and Porikos studies remains the subject of conjecture. The design differences including the snack model, subject diet awareness, and the amount of energy dilution are possible answers. It is not the first time that studies of the effects of caloric dilution in humans have given different results[22]. Further studies may provide an understanding of these effects.

Beyond the use of SPE as an energy diluent, it may have an equally important role as a means for shifting diet composition from one that is high in energy in absorbable fat to one that is low. Schaefer *et al.* reported the benefits of a low-fat diet in the treatment of hypercholesterolemic patients[25]. Anderson has reported the value of low-fat, high complex carbohydrate diets in the treatment of maturity-onset diabetes[1]. The AHA and ADA have recommended a reduction in fat intake, yet the consumption trend in the USA is toward increased fat energy. One use of SPE may be to provide a palatable means of achieving a high-carbohydrate diet. One possible result of a high-carbohydrate, low-energy density diet may be a shift toward a 'low energy balance'. Puska *et al.* reported reduced energy intake when dietary fat decreased from 108 to 52 g/d[23].

For many people a desire for the taste and lubricity of fatty foods has been instilled by culture and habit. This requirement for fat makes a significant shift towards decreased fat in the diet virutally impossible. SPE may provide the sensory perception of fat without the energy to allow a palatable means of achieving the reduced-fat, reduced-energy diet.

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Sucrose polyester: substitution for dietary fats in hypocaloric diets in the treatment of familial hypercholesterolemia[1-4]

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ABSTRACT In five obese women heterozygous for familial hypercholesterolemia, we assessed the combination of weight loss and sucrose polyester (SPE) in lowering low-density lipoprotein cholesterol (LDLC). After a 10-day basal hypocaloric (1426 cal/day), 270 mg cholesterol, P/S 1.2:1 diet, an average of 36 g of dietary fat/day was replaced by 36 g of an 80/20 SPE-hydrogenated palm oil mixture, providing 30 g SPE for 30 days; during the SPE substitution period mean dietary cholesterol and P/S were unchanged, mean caloric intake was 1104 cal/day. During the hypocaloric basal diet, mean weight fell 1.2 kg, $p < 0.02$, total plasma cholesterol fell 8% from 358 ± 46 to 330 ± 47 mg/dl, $p < 0.01$, LDLC fell 4% from 264 ± 37 to 254 ± 44 mg/dl, $p > 0.1$, and mean high-density lipoprotein cholesterol fell 11%, from 52 ± 4 to 46 ± 4, $p < 0.05$. Over the 30-day SPE substitution, mean cholesterol fell 20% from 330 ± 47 at the end of the basal diet to 265 ± 42 mg/dl, $p < 0.001$; mean LDLC fell 23%, from 254 ± 44 to 195 ± 41 mg/dl ($p < 0.01$); weight fell 4%, $p < 0.01$, from 91 ± 7 to 87 ± 7 kg, and mean high-density lipoprotein cholesterol fell 11% from 46 ± 4 to 41 ± 2, $p < 0.05$. Hypocaloric removal of dietary fat by SPE, an artificial fat with culinary properties of conventional dietary fats, effectively reduces LDLC (by 23%) in familial hypercholesterolemia subjects, with additive effects of SPE and weight loss. *Am J Clin Nutr* 1983;37:347–354.

KEY WORDS Sucrose polyester, familial hypercholesterolemia, weight loss

Introduction

Sucrose polyester (SPE), a synthetic lipid formulated from sucrose and long chain fatty acids (1), has physical, culinary, and organoleptic properties similar to those of dietary fats (2), but is totally nonabsorbed (3–5). SPE produces a persistent oil (organic) phase in the small intestine which can extract lipophilic compounds and reduce their absorption, including cholesterol (6), vitamin A (7), and DDT (8). By interfering with the micellar absorption of cholesterol from both dietary and biliary sources, SPE reduces plasma total and low-density lipoprotein cholesterol (LDLC) levels in normal subjects (5, 9, 10). SPE also provides satiety (with concomitant 10% lowering of plasma cholesterol and 14% lowering of LDLC) in normocholesterolemic obese subjects when covertly used as a replacement for dietary fat (11).

The hypocholesterolemic effect of SPE has primarily been studied with SPE as an additive to an isocaloric diet (5, 9, 10, 12). Significant mean reductions in plasma LDLC (10 to 19%) resulted from the addition of SPE to the diets of normocholesterolemic subjects (10). The addition of SPE to the diet of heterozygous familial hypercholesterolemic (FH) patients, however, produced no significant changes in plasma lipids in a 10-day period (5), and a statistically significant but small reduction in an 8-wk period (12).

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[3] This study followed a protocol approved by the University of Cincinnati Faculty Committee on Human Research.

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Our specific aim in this study was to assess the effectiveness of substitution of SPE for dietary fat as a regimen to lower total and LDLC in subjects with FH, as inpatients, utilizing metabolic diets in the Cincinnati General Clinical Center. Since it had been reported that a drastic reduction in the intake of fat was an effective treatment for hypercholesterolemic patients (13), we hypothesized that the replacement of ordinary dietary fat with SPE might provide a palatable means of achieving a low-fat diet, and that a hypocaloric diet plus SPE would represent effective therapy for lowering the levels of total and LDLC in FH subjects.

Methods

Patients

The protocol followed had been approved by the University of Cincinnati Medical Center Committee on Human Experimentation.

Five women with well-defined FH who were at least 20% above ideal body weight, and had no concurrent secondary causes of hypercholesterolemia, were studied. Four of the five women had tendon xanthomas, all five had arcus juvenalis, and two had periorbital xanthelasmas. All five women had one or more first-degree relatives with FH. Table 1 describes the five patients at entry into the study. Patients were required to be free of angina, tachycardia, and evidence of remote or recent myocardial infarction on entry. Patients were required to be free of gastritis, pancreatitis, cholecystitis, and recurrent gastroenteritis. Concomitant therapies including thyroid hormone, estrogens, progestins, anorectic agents, lipid-lowering agents, laxatives, digoxin, coumadin-like preparations, and blood pressure lowering medications were cause for exclusion.

SPE formulation

SPE was prepared from safflower oil fatty acids by a solvent free process (1). It was mixed with completely hydrogenated palm oil (HPO) in a ratio of 80 SPE:20 HPO by weight to provide stiffening of the fecal fat and prevent anal leakage of the SPE oil. In order to provide 30 g of SPE/day, an average of 36 g of the 80/20 SPE/HPO mixture was substituted for 36 g of conventional dietary fat.

Dietary intake during basal and SPE substitution periods, and study design

Patients began the study with a hypocaloric 10-day basal diet with a mean P/S of 1.2:1, 40% of calories as fat, and 269 mg/day dietary cholesterol (**Fig 1, Table 2**). During the 30-day SPE substitution period (Fig 1), mean dietary cholesterol and P/S were maintained at 265 mg and 1.1:1, respectively, not significantly different from the ten day basal diet ($p > 0.1$). An average of 322 kcal/day from conventional dietary fat were deleted from the diet by substitution of 36 g SPE-HPO mix for 36 g of conventional fats (Table 2). Based on previous studies (11), the HPO which accompanied the SPE in the SPE-HPO mixture was poorly absorbed because of its high melting point. The HPO fatty acid composition was 41% palmitic, 57% stearic, and 2% other fatty acids. Our study thus allowed assessment of the LDLC lowering properties of fat and calorie restriction, without confounding by simultaneous major changes in dietary cholesterol and P/S ratios (Fig 1, Table 2). The removal of dietary fat during the SPE period resulted in mean decrements in dietary vitamin A and E of 16 and 26%, respectively, by removal of vegetable oils and fat containing A and E (Table 2). Table 2 displays the dietary regimen for the individual subjects.

Safety and efficacy evaluation

Blood samples were drawn after a 12-h fast on the first 3 and final 3 days of the basal period, and on the final 3 days of each succeeding 10-day segment during the treatment period (Fig 1, Table 2). In some subjects samples from the first and final 4 days of the study periods were obtained. Measurements of plasma lipids and lipoproteins (14) and clinical chemistries were obtained at each blood sampling (Fig 1, Table 2). Vitamins A and E in plasma were measured according to previously published methods (15, 16). Subjective responses to palatibility and acceptability of the diet were elicited from patients by questionnaires. The effect of SPE on bowel movements and stool consistency was also determined by written questionnaire.

Statistical methods

Two way analysis of variance (17) (patients by treatments) was used to compare the initial and final 3-day segments of the basal period and to determine the treatment effect of SPE compared to the final basal period values in subjects with SPE substitution (**Tables 3 to 5**). Relationships between changes in weight and plasma lipids and lipoproteins were assessed using Spearman's rank order correlations (17).

TABLE 1
Plasma total, LDLC, HDLC, and VLDLC, and triglyceride (TG) levels (mg/dl) in five female patients on entry into the study

Subject	Age	Cholesterol	LDLC	HDLC	VLDLC	TG	Wt
	yr						*kg*
1	51	529	398	55	76	379	74.7
2	29	349	241	59	49	246	114.3
3	46	276	195	58	24	118	84.4
4	60	276	202	49	24	121	104.8
5	54	359	286	37	36	179	83.3

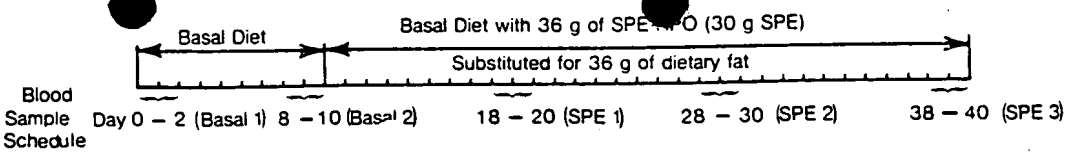

FIG 1. Study design for SPE substitution.

TABLE 2
Dietary intake during basal and SPE substitution periods

Subject	Basal	SPE	Basal	SPE	Basal	SPE
	Cal/day		% Cal as Fat		Vitamin A (IU/day)	
1	1497	1210	36%	20%	5542	4552
2	1325	1037	36%	19%	4379	3389
3	1336	978	44%	23%	7826	7165
4	1642	1285	43%	27%	5216	4391
5	1328	1009	42%	25%	4038	3206
x̄ (SEM)	1426 ± 63	1104 ± 61	40 ± 2	23 ± 1	5400 ± 665	4541 ± 708
	Basal	SPE	Basal	SPE	Basal	SPE
	Vitamin E (mg/day)		Cholesterol (mg/day)		P/S	
1	10.6	7.4	266	266	1.2:1	.9:1
2	7.5	4.3	281	281	1:1	.5:1
3	26.9	22.7	278	278	1.5:1	1.3:1
4	18.3	13.5	263	263	1.2:1	1.3:1
5	6.7	4.0	257	239	1.2:1	1.4:1
x̄ (SEM)	14.0 ± 3.8	10.4 ± 3.5	269 ± 4.5	265 ± 7.4	1.2 ± .08	1.1 ± .17

Results

Plasma lipids and lipoproteins

The use of SPE-HPO mix as a substitute for dietary fat resulted in consistent significant decreases from the final basal period values for the group of five FH subjects for plasma total and LDLC (Table 3, Fig 2).

During the basal hypocaloric diet period, total plasma cholesterol fell 8% ($p < 0.01$), accompanying a fall in mean weight of 1.2 kg ($p < 0.02$). From the end of the basal diet period to the end of SPE substitution, total plasma cholesterol fell 20% (Table 3, Fig 2), $p < 0.01$.

Comparing the values at the beginning of the basal period with the values at the end of the basal period for LDLC in the group of five subjects, mean LDLC fell only by 4% ($p > 0.1$). Comparing mean LDLC for the group after 30 days on SPE with that at the end of the final basal period, LDLC fell 23% ($p < 0.01$) (Table 3, Fig 2). Mean weight fell 4 kg during the 30-day SPE substitution period, $p < 0.01$ (Table 5).

There was a significant fall in mean high-density lipoprotein cholesterol (HDLC) from the beginning of the basal diet period to the end of the basal period (11%, $p < 0.05$, Table 3). Moreover, comparing mean HDLC at the final basal period with that at the end of SPE treatment 3, mean HDLC levels had fallen an additional 11% ($p < 0.05$, Table 3).

Despite a mean 29% reduction in very low-density lipoprotein cholesterol (VLDLC) going from the initial basal period to the final basal period, this within-basal change was not significant ($p > 0.1$). There were no significant changes in VLDLC in the SPE substitution period.

Vitamin A and E

Plasma Vitamin A and E levels did not fall significantly during the basal period. Subsequently, however, vitamin A levels were significantly lower during the three SPE substitution periods (Table 4), and vitamin E levels were lower, comparing values at the end of the basal period with those at the end of the second SPE period, and those at the end of the third SPE period (Table 4). The major decreases in plasma vitamin A and E occurred during the first 10-day SPE period, and levels of A and E remained relatively

TABLE 3
Mean* plasma total cholesterol, HDLC, LDLC, VLDLC (mg/dl) by subject and by treatment period

Subject	Total cholesterol					HDLC				
	Initial basal 1	Final basal 2	SPE1	SPE2	SPE3	Initial basal	Final basal	SPE1	SPE2	SPE3
1	529	510	465	456	426	55	52	39	34	38
2	349	312	233	223	212	59	51	47	43	45
3	276	262	225	204	199	58	46	48	48	45
4	276	254	227	218	216	49	49	48	48	43
5	359	312	280	268	269	37	33	34	33	32
$\bar{x}$ ± SEM	358 ± 46†	330 ± 47	286 ± 46‡	274 ± 47§	265 ± 42 ‖	52 ± 4¶	46 ± 4	43 ± 3 NS	41 ± 3**	41 ± 2††
	LDLC					VLDLC				
1	398	422	390	380	352	76	37	36	42	36
2	241	236	162	152	143	49	25	24	27	24
3	195	185	152	132	131	24	32	25	25	24
4	202	184	158	151	150	24	21	20	20	23
5	286	245	209	199	201	36	21	37	36	36
$\bar{x}$ ± SEM	264 ± 37 NS	254 ± 44	214 ± 45‡	203 ± 46§	195 ± 41 ‖	42 ± 10 NS	30 ± 3	28 ± 3 NS	30 ± 4 NS	29 ± 3 NS

* Mean of last three values during basal and SPE treatment periods.
† $p < 0.01$ (initial basal vs final basal).
‡ $p < 0.01$ (final basal vs SPE 1).
§ $p < 0.01$ (final basal vs SPE 2).
‖ $p < 0.01$ (final basal vs SPE3).
¶ $p < 0.05$ (initial basal vs final basal).
** $p < 0.05$ (final basal vs SPE 2).
†† $p < 0.05$ (final basal vs SPE 3).

TABLE 4
Mean* plasma vitamin A (μg/dl) and E (mg/dl) by subject and treatment period

Subject	Basal 1	Basal 2	SPE 1	SPE 2	SPE 3
1 A	156.1	161.0	112.5	118.1	97.6
E	3.33	2.68	1.94	1.80	1.64
2 A	66.5	53.9	40.6	34.4	38.0
E	1.48	1.04	0.71	0.75	0.81
3 A	52.3	53.4	40.4	38.4	41.4
E	0.80	1.18	0.80	0.79	0.61
4 A	31.7	23.7	24.0	23.4	23.1
E	1.93	1.95	1.55	1.49	1.60
5 A	57.1	58.9	56.8	51.9	59.3
E	1.71	1.60	1.22	1.29	1.41
A $\bar{x} \pm$ SEM	72.7 ± 21.6 NS	69.0 ± 23.7	55.3 ± 15.2†	53.2 ± 16.8‡	52.0 ± 12.7§
E	1.85 ± .42 NS	1.69 ± .30	1.24 ± .23 NS	1.22 ± .20‡	1.21 ± .21 ‖

* Mean of last three values during basal and SPE treatment periods.
† $p < 0.02$ (final basal vs SPE 1).
‡ $p < 0.02$ (final basal vs SPE 2).
§ $p < 0.02$ (final basal vs SPE 3).
‖ $p < 0.01$ (final basal vs SPE 3).

TABLE 5
Mean* body wt (kg) by subject and by treatment period

Subject	Basal 1	Basal 2	SPE 1	SPE 2	SPE 3
1	74.7	73.5	72.3	71.3	70.1
2	114.3	112.3	110.4	108.1	106.4
3	84.4	83.3	81.8	81.4	80.8
4	104.8	104.1	102.6	102.4	101.4
5	83.4	82.7	80.9	80.1	78.9
$\bar{x} \pm$ SEM	92.3 ± 7.4†	91.2 ± 7.3	89.6 ± 7.2‡	88.7 ± 7.0§	87.5 ± 7.0 ‖

* Mean of last three values during basal and SPE treatment periods.
† $p < 0.02$ (initial basal vs final basal).
‡ $p < 0.02$ (final basal vs SPE 1).
§ $p < 0.01$ (final basal vs SPE 2).
‖ $p < 0.01$ (final basal vs SPE 3).

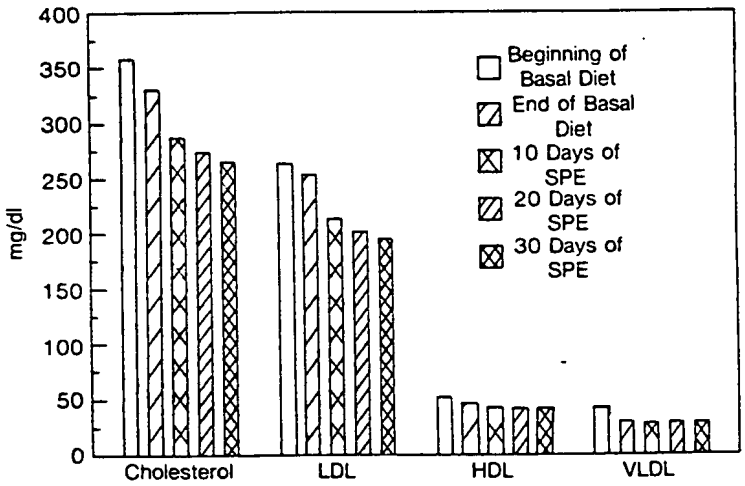

FIG 2. Mean levels of total, LDL, HDL, and VLDL cholesterol on a 10-day basal hypocaloric diet and after 10, 20, and 30 days of SPE substitution, 30 g/day.

constant during the next 20-day period (Table 4). There were no significant correlations between changes in plasma vitamin A and E levels during SPE substitution and changes in either lipoproteins or body weight.

An indicator of vitamin K status, the partial thromboplastin time, remained constant throughout the study.

Body weight

As shown in Table 5, there was significant weight loss between the beginning and end of the hypocaloric basal diet period, and between the final basal period and each of the SPE treatment periods. Mean weight loss for the group, comparing the final SPE period with the final basal period, was 3.7 kg, ($p < 0.01$).

There were no significant associations between changes in body weight and changes in lipids and lipoproteins during the basal period, or between changes in body weight and lipoproteins between the end of the basal period and the end of three 10-day periods on SPE. Overall, the decrements in body weight during the SPE substitution period and the decrements in LDLC during this period were positively, but not significantly correlated, $r = 0.5$ to 0.7, ($0.05 < p < 0.1$).

Blood chemistry

There were small, clinically insignificant decreases in Hb, hematocrit, blood urea nitrogen, and phosphorous during the SPE substitution period. Mean (SEM) fasting blood glucose was 93 ± 5 mg/dl at the end of the basal period and fell to 82 ± 3 mg/dl at the end of the SPE substitution, $p < 0.05$. This decrease in fasting blood glucose in obese, nondiabetic subjects is similar to improvement in peripheral glucose handling and increased sensitivity to insulin seen during weight loss in obese, diabetic subjects (18).

Adverse reactions

Questionnaires were used to solicit comments from subjects to determine any clinical or physical changes that might be attributed to SPE. Three of the subjects reported increases in soft stools, and transient oily anal residue of the SPE after defecation, compared to the basal diet period.

Discussion

This report focused on the effectiveness of substitution of SPE for dietary fat as a regimen to lower LDLC in FH subjects. The removal of fat (primarily saturated fat) from the diet has been reported to be an effective means of reducing total and LDLC (13). During the SPE substitution, the mean daily intake of cholesterol and the dietary P/S ratio were both maintained, so that they did not differ significantly from the 10 day basal diet, thus allowing an assessment of the effects of reducing total caloric and total fat intake without confounding by changes in dietary composition. In the substitution phase of this study we removed an average of 36 g/day of dietary fat from the diet, replacing it with a nonabsorbable fat-like material, SPE. Compared to a hypocaloric diet without SPE, substitution of dietary fat (and calories) with SPE led to mean reductions of LDLC of 23%, and of weight (4.1%, 3.7 kg). We also observed a significant decrease in HDLC, most marked in two of the five FH women, about half of the relative decrease in LDLC.

Our results suggest that reduction of cholesterol absorption by SPE coupled with hypocaloric diet (with weight reduction) facilitate substantial LDLC reduction in FH subjects. A similar combined effect between a hypocholesterolemic drug, colestipol resin, and weight loss has recently been shown by Davis et al (19). In five FH patients receiving colestipol, with a mean weight loss of 7.7 kg, plasma total cholesterol and LDLC were reduced 10 and 9% beyond effects of colestipol alone, while HDLC increased (19). The subjects' sex was not specified (19).

There have been conflicting reports of the effects of weight loss on plasma lipoproteins (20–23). Generally, weight loss reduces triglyceride and VLDLC levels (20–23), and may elevate HDLC levels in men. Weight loss in obese women may, however, lower HDLC (21). In the five FH women in the current study, HDLC fell significantly during the basal hypocaloric diet period, and fell further in the more hypocaloric, 30-day SPE substitution period. Isocaloric diets with SPE addition have not been associated with significant reduction in HDLC (5, 10). We speculate that the HDLC decrement in the five

FH women during the hypocaloric SPE substitution period is primarily attributable to weight loss, although reduction in dietary cholesterol absorption may also have contributed to reduction in HDLC. There have been no consistent reports, however, showing that reductions in LDLC in hypercholesterolemic subjects result from weight loss alone.

In this study the mean weight reduction of the five women during the basal hypocaloric diet period was greater than 1 kg. This basal period weight loss was accompanied by significant reductions in total and HDLC. Most of the total cholesterol decrease during the basal, hypocaloric diet period was accounted for by decrements in the VLDLC and HDLC. The reduction in LDLC was not significant during this period of weight loss. The first 10 days of the SPE substitution period produced the greatest decrease in plasma LDLC. After completion of the study, and after cessation of SPE, as outpatients, the subjects were unable to maintain their new and lower body weight, thus obviating a further possibility of differentiating between independent hypercholesterolemic effects of weight loss and SPE.

The lack of significant, consistent lowering of LDLC by SPE substitution for dietary fat in our previous study of two hypercholesterolemic subjects (5) may have been the result of the high (800 mg/day) cholesterol level included in the diet. In the study reported here, the dietary cholesterol level was maintained stable throughout at a lower level of 265 mg/day. An interaction of SPE with the hypocaloric diet, similar to a colestipol-weight loss interaction (19), may also explain the large reductions in LDLC in the study described here. During the SPE substitution period, there were positive, but weak correlations between the degree of weight loss and the reduction in LDLC.

SPE's effect on plasma levels of vitamin A and E may, in part, be accounted for by the reduction in dietary vitamins A (16%) and E (26%) that accompanied the replacement of triglyceride fats. Moreover, since vitamin E is transported by both HDL and LDL (10, 24), significant reductions in HDL and LDL might lead to an apparent decrease in plasma vitamin E (seen in 10 days), but probably do not reflect a decrease in body stores of vitamin E. The rapid appearance of a decrement in vitamin A (within 10 days), coupled with a decrement in LDLC, again suggests a transient diminution of plasma vitamin A levels, but is unlikely to reflect a decrease in body pool levels. It is also possible that SPE reduces the absorption of phytofluenes, which, despite correction measures (15), may augment actual plasma vitamin A fluorescence. Hence, we speculate that a SPE-mediated reduction in "nonvitamin A" fluorescence from phytofluenes, might account, in part, for the apparent decrease in vitamin A levels. Nevertheless there is an apparent effect of the SPE itself on plasma levels of these vitamins, since the addition of SPE to the diets effected reductions of plasma vitamins A and E, in this and in other studies (5, 10). Presumably, the retention of these fat-soluble vitamins in the intestinal SPE oil phase is a partial causative factor, leading to reduction in plasma vitamin A and E levels.

The reduction of plasma vitamin A and E occurred primarily in the first 10-day exposure to SPE. After that period, the plasma levels reached an apparent plateau. Extrapolation of these values would suggest that a reduction in fat-soluble vitamin status by SPE may be a limited effect. There were no decreases of vitamins A and E below normal limits in any subject.

As shown in a placebo-controlled study with hypercholesterolemic outpatients, the addition of SPE to the diet can produce small reductions in total and LDLC (12). In the study reported here, however, we have found that clinically significant reductions in total and LDLC are achieved by the use of SPE as a substitute for conventional dietary fat, allowing the removal of fat and calories from the diet in a palatable manner.

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The rate of energy storage in adipose tissues is, at least in part, dependent on the concentration of nutrients and hormones in the blood. A delayed absorption of ingesta from the intestine should cause lower concentrations of fat, glucose and insulin and, thus, reduce the triglyceride storage rate.

Non-selective agents retard the absorption irrespective of composition of food. Inhibitors of intestinal α-glucosidases delay the degradation of complex carbohydrates to absorbable monosaccharides and thus decrease the rate of their absorption. Inhibitors of pancreatic lipase interfere with the degradation of dietary triglycerides and decrease the postprandial triglyceride increment in blood and tissues. Recently a compound was found which inhibits the absorption of carbohydrates as well as triglycerides.

This review will focus on compounds which alter the intestinal absorption of nutrients, in particular the absorption rate of carbohydrates and lipids. Non-selective agents as well as specific inhibitors of carbohydrate and lipid absorption with different mechanisms of action will be described.

Non-selective agents

Delay of nutrient absorption has been reported with perfluorooctyl bromide (PFB)[16] which coats the stomach and intestine. Administration of PFB brought about a reduced gain in body weight of meal-fed rats.

Administration of (±)-*trans*-epoxyaconitate[45] or (−)-*threo*-chlorocitric acid[46,47] decreased food intake, body weight gain and total body lipids in rats. This anorectic effect appeared to be related to a reduction in the rate of gastric emptying.

Recently, it was reported that biguanides, which inhibit intestinal glucose absorption[11], also delay the absorption of triglycerides[9]. No hypothesis was given for the possible mechanisms of action.

Different types of plant fibres, eg guar gum, mannan etc., delay the absorption of carbohydrates[19,20] and fats[17] in man. In feeding experiments on rats dietary fibre (10 g/100 g food) brought about a significantly reduced body weight gain[28]. The effects of dietary fibres appear to be related to their physicochemical properties[53]. This may also be true for the effect of fibres on the digestive enzymes of the pancreas *in vitro*[18].

Inhibitors of carbohydrate absorption

Inhibitors of glucose absorption

Phlorizin and biguanides[11] reduce the absorption rate of glucose and biguanides have been reported to reduce body weight gain in man[30]. This effect has been attributed to a reduced absorption of glucose from the intestine[13,44] or to an anorectic effect[31] induced by these compounds.

Inhibitors of α-amylase

Since dietary di-, oligo- or polysaccharides must be degraded to monosaccharides by intestinal glucosidases, eg amylase, before they are absorbed, the inhibition of these enzymes

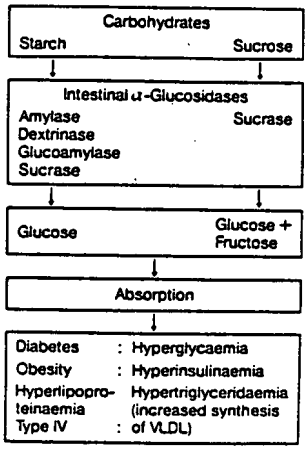
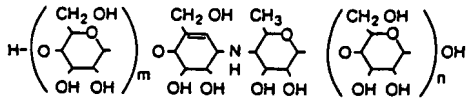


Fig. 1. *Concept of α-glucosidase inhibition*



BAY e 4609: m + n ≈ 7–30 glucose units

Mol. W. : ≈ 1500–6000

Inhibitory activity : 3300 AIU (FIP)/mg

Fig. 2. *General formula of α-amylase inhibitors in BAY E 4609.* AIU = amylase inhibitory unit, FIP

should cause a reduced carbohydrate absorption rate (Fig. 1). As a consequence a diminished increase of postprandial glycaemia, insulinaemia, and secretion of very-low-density lipoproteins (VLDL) from gut wall and liver was expected[32].

Most inhibitors of pancreatic α-amylase have been extracted from various kinds of grain or culture filtrates of micro-organisms[48]. They are proteins, glycoproteins or pseudo-oligosaccharides and inhibit mammalian α-amylase *in vitro*. The first α-amylase inhibitor (BAY D 7791) which was tested in appropriate carbohydrate loading tests in animals and man[32] was isolated by Schmidt[39] from wheat flour and found to be a protein.[49] Administration of BAY D 7791 retarded the intestinal digestion of starch from 2 to > 4 h and reduced the postprandial blood glucose and serum insulin increments in starch loading tests on rats, dogs and man[32]. A 50 per cent reduction of the postprandial blood glucose increment was achieved by administration of approximately 50 mg BAY D 7791/kg rat or 500 mg/person. Oral administration of 214 mg/kg rat was not effective in loading tests using *cooked* instead of *raw* starch. There was no transport of undigested raw starch into the colon of rats although very high doses of this inhibitor had been administered.

In 1975 the first data were reported on phaseolamin[29], a glycoprotein isolated from kidney beans, which inhibits α-amylase *in vitro*. Administration of phaseolamin (1000 mg/person) in man immediately before a high-starch test meal did not affect the energy content of the ingesta or faeces[3]. No previous data had been reported with regard to the effects of phaseolamin on the postprandial blood glucose and serum insulin in loading tests in animals or man consuming raw or cooked starch. Recently a protein was found in the culture broth micro-organisms (HOE 467, Tendamistat) which inactivates mammalian α-amylase[52]. In starch loading tests this amylase inactivator reduced the postprandial increase of blood glucose and serum insulin concentrations in rodents[38]. A series of pseudo-oligosaccharides exerting amylase inhibitory activity has been reported, eg BAY E 4609, amylostatin, TAI A, TAI B and trestatin A, B, C (for review see[40,48]). They were isolated from the culture filtrates of actinomycetes or streptomyces. The general formula of one of these inhibitors, BAY E 4609, is shown in Fig. 2. The molecular core, which is essential for the inhibitory activity, is composed of an unsaturated cyclitol unit and a 4-amino-4, 6-dideoxy-*D*-glucopyranose unit[40]. The core is linked to 7-30 glucose residues in $m + n$. The structure of the other pseudosaccharidic amylase inhibitors mentioned above is very similar to this structure (for review see[48]).

As with BAY D 7791, the dose of BAY E 4609 which reduced the postprandial blood glucose increment in starch loading tests on rats by 50 per cent in comparison to control animals (ED_{50}), was much higher in loading tests using *cooked* instead of *raw* starch[36]. The average ED_{50} was 20 000 and 1600 amylase inhibitory units (AIU) of BAY E 4609/kg rat, respectively. In contrast to BAY D 7791, overdosage of BAY E 4609, ie more than 4000 AIU/kg rat, brought about a significant transport of undigested raw starch into the large intestine. Lower doses did not exert this effect.

In feeding experiments on meal-fed rats, administration of BAY E 4609 brought about a reduced conversion of glucose from ^{14}C-labelled starch into lipids of epididymal and subcutaneous adipose tissues as well as into lipids of aortic tissues[25,36]. This effect appeared to be related to a reduced postprandial insulin increment as was shown in acute loading tests. In 45-day feeding experiments addition of BAY E 4609 to standard food dose-dependently reduced the body weight gain and carcass lipid content of genetically-obese rats. Since BAY E 4609 was devoid of sucrase inhibitor activity it was ineffective in loading tests on rats and man when the disaccharide sucrose was administered in addition to starch[36] (Table 1).

Table 1. *Effect of BAY E 4609 on body weight gain, protein and lipids in the carcass of genetically-obese 'Zucker' rats (fa, fa) after 45 d feeding 0.25 or 0.5 mega AIU BAY E 4609/100 g standard food (upper part) and 35 d feeding 0.5 mega AIU/100 g sucrose containing diet (lower part)*

Standard food (45 d)	*Body weight (g)*		*Carcass (g)*	
	initial	*gain*	*protein*	*lipids*
Lean rats (*fa*,-)	225	85 ± 13	63 ± 17	15 ± 5
fa, fa; control	328	164 ± 19	60 ± 7	149 ± 19
fa, fa, 0.25 mega AIU	303	137 ± 21*	53 ± 10	123 ± 13*
fa, fa, 0.50 mega AIU	300	97 ± 27*	50 ± 11	89 ± 18**
Sucrose containing diet (35 days)				
fa, fa, control	373	128 ± 62	45 ± 3	149 ± 26
fa, fa, 0.5 mega AIU	360	109 ± 51	44 ± 8	147 ± 13

*$P < 0.05$, **$P < 0.01$ compared to control.

Cycloleucine (1-amino-1-cyclopentane carboxylic acid)[1] is, chemically, completely different from the above-mentioned α-amylase inhibitors. Administration in food gave rise to a reduced body weight gain by decreasing the efficiency of food utilization without effect on food consumption. The activities of sucrase, maltase, lactase and pancreatic lipase were not significantly affected. There are no data available which give evidence for an inhibitory effect on α-amylase *in vitro*. The α-amylase activity in the pancreas of rats was dose-dependently diminished after 3 d of feeding this agent. The reduced food efficiency might be related to a reduced secretion of pancreatic amylase or reduced biosynthesis of this enzyme or to both.

Inhibitors of single disaccharidases

Examples of selective disaccharidase inhibitors are TRIS (tris(hydroxymethyl)aminomethane) and SaH 50-283 (2,2-dimethyl-1-(4-methylphenyl)-1-propanone). TRIS was reported to inhibit intestinal sucrase and the digestion of sucrose in oral loading tests on rats and man[12,33]. SaH 50-283 was reported to inhibit intestinal maltase activity[15]. The ED_{25} for lowering postprandial blood glucose increment in maltose loading tests on rats was calculated to be 12 mg/kg body weight. Food efficiency in meal-fed rats was significantly lowered which could be partially accounted for by maltase inhibition.

Inhibitors affecting more than one glucosidase

Agents which inhibit a broad spectrum of intestinal α-glucosidases involved in the degradation of starch and sucrose are summarized in Fig. 3. The chemical structures have been comprehensively reported[40,48]. Some of them are polypeptides, but most inhibitors are pseudo-oligosaccharides as shown in Fig. 2.

Origin	Chemistry	Code	Inhibition of digestion: Starch	Sucrose
Streptomyces (1966)	Nojirimycin		+	+
Streptomyces (1966)	1-Deoxynojirimycin		+	+
Actinoplanes (1970)	Pseudotetrasaccharide	Acarbose	+	+
Streptomyces (1976)	Glycopeptide		+	+
Streptomyces (1979)	Pseudooligosaccharide	SF-1130-X_1	+	+
		SF-1130-X_2	+	+
		SF-1130-X_3	+	+
Semisynthetic	Derivatives of deoxynojirimycin	BAY m 1099	+	+
		BAY o 1248	+	+
Semisynthetic	N-Alkyl derivatives of Valienamin, Valiolamin, Validamin	Takeda	?	?

Fig. 3. *α-glucosidase inhibitors affecting the intestinal degradation of starch and sucrose*

The glucosidase inhibitor investigated most thoroughly is acarbose (Fig. 4). It has a rather weak inhibitory activity on pancreatic amylase, but a strong inhibitory effect on the activity of maltase, sucrase, glucoamylase and dextrinase[35]. Acarbose was reported to inhibit mammalian sucrase competitively[4,41]. The affinity of acarbose for sucrase was 10^4-10^5 times greater than that of sucrose for this enzyme. In carbohydrate-loading tests the postprandial increments of blood glucose and serum insulin concentrations were dose-dependently reduced. The ED_{50} was 0.5-1.5 mg acarbose/kg body weight in rats as well as in man[34]. After oral administration of ^{14}C-labelled sucrose acarbose brought about a reduced conversion of sucrose into the lipids of the perirenal and epididymal adipose tissue of meal-fed rats[37]. When acarbose was administered to meal-fed rats in a mixed meal containing ^{14}C-labelled triolein and unlabelled carbohydrates, olive oil and casein the incorporation of radioactivity

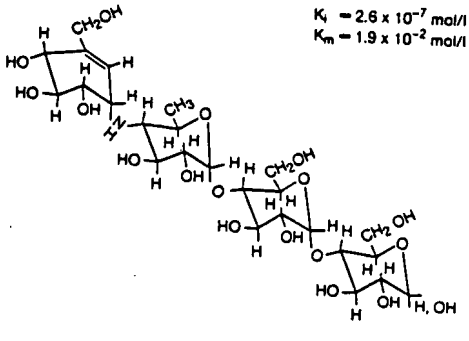


Fig. 4. *Structure of acarbose (BAY G 5421)*[41]

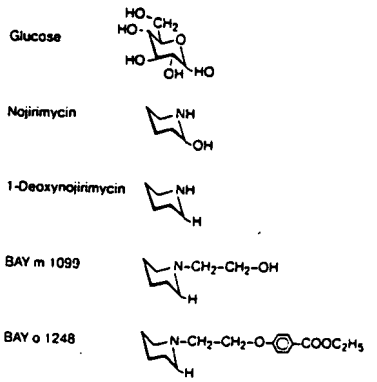


Fig. 5. *Structure of glucose, nojirimycin, 1-deoxynojirimycin, BAY M 1099 and BAY 0 1248*

Table 2. *Effect of 8 d refeeding with and without 50 mg acarbose (BAY G 5421)/100 g feed on mean (± s.e.) liver and muscle glycogen and epididymal and perirenal fat pads of rats*

	Glycogen (mg/whole tissue)		*Fat pads* (g/animal)	
	Liver	*M. tib. ant.*	*Epididymal*	*Perirenal*
Controls, fed *ad lib.*	409 ± 48	1.64 ± 0.42	2.21 ± 0.21	1.86 ± 0.24
Fasted	12 ± 6	0.32 ± 0.08	0.52 ± 0.14	0.21 ± 0.13
Refed, control	503 ± 39	1.70 ± 0.16	1.73 ± 0.11	1.29 ± 0.21
Refed with acarbose	377 ± 47*	1.75 ± 0.13	1.26 ± 0.06**	0.66 ± 0.04**

*$P < 0.05$, **$P < 0.01$ vs refed controls (Björntorp *et al.*, 1983).

into the perirenal adipose tissue was significantly delayed. In refeeding experiments, on previously fasted rats, acarbose decreased the regain of perirenal and epididymal fat pads significantly (Table 2)[2]. It was suggested that these effects were not related to carbohydrate malabsorption but rather to reduced postprandial serum insulin concentrations. Feeding of 20, 40 or 80 mg acarbose/100 g semisynthetic diet for 34 weeks resulted in a dose-dependent reduction in body weight gain[35]. This effect was more marked in young, genetically-obese rats than in Wistar rats. In adult, genetically-obese rats a reduced gain of body weight or a loss in body weight induced by administration of 20 or 40 mg acarbose/100 g of diet was found to be related to a dose-dependent reduction of food consumption. Administration of a higher dose (80 mg/100 g diet) was associated with diarrhoea due to carbohydrate malabsorption. A marked reduction of hypertriglyceridaemia was reported in genetically-hyperlipoproteinaemic 'Zucker' rats and in normal rats receiving a fat-free diet[26,35,54] This effect was due to a reduced secretion of VLDL.

As was expected from animal experiments and clinico-pharmacological studies[34] administration of acarbose improved the metabolic status of patients suffering from diabetes mellitus, Type I or Type II, as was shown by reduced hyperglycaemia and glucosuria[8].

It was reported recently that nojirimycin and 1-deoxynojirimycin are glucosidase inhibitors[40,42]. The deoxynojirimycin derivatives BAY M 1099[21] and BAY 0 1248[22] (Fig. 5) are also potent glucosidase inhibitors. But in contrast to acarbose they are almost completely absorbed from the intestines of rats. Overdosage of these α-glucosidase inhibitors did not cause carbohydrate maldigestion in rats, as assessed by faecal carbohydrate excretion and intestinal gas volume. Neither of these inhibitors affected the activity of pancreatic amylase *in vitro*. The inhibitor constants (K_i) using intestinal sucrase from hogs ($K_m = 1.8 \times 10^{-2}$ mol/l) and sucrose as substrate were 1.4×10^{-7} and 0.4×10^{-7} mol/l, respectively. The affinity of these inhibitors for intestinal maltase and isomaltase was also approximately 10^5 times greater than that of their natural substrates maltose and isomaltose. The main pharmacological properties of these inhibitors are shown in Figs 6 and 7. The main pharmacological difference between the two inhibitors is the duration of action. Whilst BAY M 1099, like acarbose, had no effect on the intestinal degradation of starch and sucrose in rats, when administered 1 h before carbohydrate loading, BAY 0 1248 was very effective even when administered 4 or 17 h before carbohydrate loading (Fig. 7). Feeding of BAY M 1099 or BAY 0 1248 brought about a reduction of food consumption, body weight gain and fat pads of rats. Clinical trials with these deoxynojirimycin derivatives have recently been initiated.

Inhibitors of lipid absorption

A decreased fat absorption rate can be induced by inhibition of pancreatic lipase activity, disturbance of micelle formation, reduced uptake of triglyceride degradation products from

■ Reduction of postprandial blood glucose and serum insulin increment in carbohydrate (CHO) loading tests on rats:

carbohydrate	ED_{50}, mg/kg body weight
glucose	> 100
sucrose	0.24
raw starch	0.48
cooked starch	0.50
cooked starch + sucrose	0.36

■ Reduction of: food intake, gain of body weight, adipose tissue, hyperinsulinaemia, and hyperlipoproteinaemia in feeding experiments on rats.

■ No excretion of undigested dietary CHO in the faeces of rats.

Fig. 6. Main pharmacological effects of BAY M 1099

■ Reduction of postprandial blood glucose and serum insulin increment in carbohydrate (CHO) loading tests on rats:

carbohydrate	ED_{50}, mg/kg body weight
glucose	> 30
sucrose	0.2
4 hours before sucrose	0.2
17 hours before sucrose	1.5
cooked starch	0.3
4 hours before cooked starch	< 1.0

■ Reduction of: food intake, gain of body weight, adipose tissue, hyperinsulinaemia, and hyperlipoproteinaemia in feeding experiments on rats.

■ No excretion of undigested dietary CHO in the faeces of rats.

Fig. 7. Main pharmacological effects of BAY 0 1248

the gut lumen, inhibition of triglyceride re-esterification or chylomicron formation in the enterocyte or a reduced release of chylomicrons from enterocytes into the lymph.

Fenfluramine decreased fat absorption rate in rats[14] probably due to an inhibition of pancreatic lipase[5,10]. Up to now there is no evidence that this effect of fenfluramine contributes to a reduction of body weight.

A hydrophobic surfactant, pluronic L-101, was reported to inhibit pancreatic lipase. The K_i was calculated to be 5.2 µmol/l[6,7]. Pluronic L-101 significantly increased the retention of tri(-1^{14}C)olein in the stomach and small intestine and the excretion of radioactivity in the faeces of meal-fed rats. The ^{14}C activity of the lipids in liver and adipose tissue was significantly decreased. After 5 d administration of pluronic L-101, the serum triglyceride concentrations in meal-fed rats were reduced. When administered in food (1 or 3 per cent) during a 42-d feeding experiment pluronic L-101 brought about a dose-dependent decrease in body weight gain and carcass lipid content in meal-fed rats without affecting food intake. Faecal excretion of dietary fat was increased. Serum concentrations of triglycerides, cholesterol and glucose remained unchanged. Pluronic F-68, a hydrophilic surface-active agent, displaying a poor lipase inhibitory activity *in vitro*, did not affect body weight gain or faecal fat excretion[7]. The effects of pluronic L-101, therefore, appeared to be related to fat malabsorption induced by lipase inhibition. Similar to pluronic L-101 the polyether BAY L 1442 was reported to inhibit pancreatic lipase *in vitro* and to reduce the postprandial increment of serum triglycerides in fat loading tests on rats[27,43]. This polyether was 10 and 75 times more potent than neomycin and cholestyramine, respectively. In contrast to pluronic L-101, a decreased postprandial serum cholesterol increase was achieved after administration of BAY L 1442 in appropriate cholesterol loading tests in rats. In 21-d feeding experiments, administration of BAY L 1442 diminished the carcass lipid content; an increased carcass cholesterol content, induced by feeding a cholesterol-enriched diet, was normalized. It was recently reported that administration of the hydrophobic surfactant pluronic L-81 leads to an accumulation of chylomicrons in the gut wall of the small intestine due to a blocked transport into the lymph[50,51]. This effect may contribute to a reduced absorption of dietary fat from the intestines.

Undesired effects like these were not observed in experiments using BAY N 4605 (*N-n*-dodecylaminocarbonyl-2,2,4-trimethyl-1,2,3,4-tetrahydrochinolin)[24] (Fig. 8). This compound inhibited pancreatic lipase from hogs *in vitro*. The K_i was approximately 2×10^{-6} mol/l. In fat loading tests on rats (2.1 g olive oil/kg body weight) administration of BAY N 4605 reduced the postprandial serum triglyceride increment dose dependently (Fig. 9). The ED_{50} was calculated to be approximately 1 mg BAY N 4605/kg rat. The incorporation of radioactivity from ^{14}C-triolein into liver, heart, adipose tissue and skin was reduced to approximately 20 per cent in comparison to control rats (Fig. 10). A 14-d administration of 300 or 1000 mg BAY N 4605/kg body weight per d by gavage did not affect the body

ucose and serum
(CHO) loading

ED_{50}, mg/kg body weight
> 30
0.2
0.2
1.5
0.3
< 1.0

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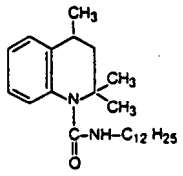
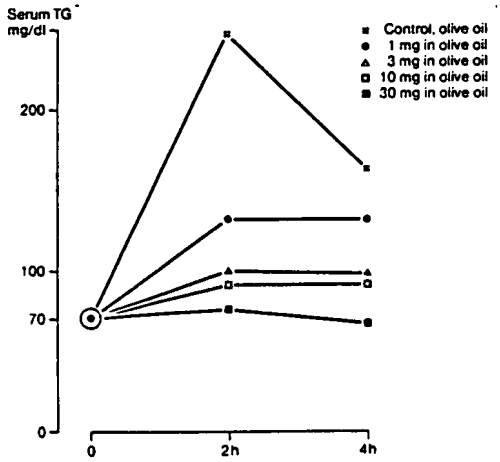


Fig. 8 (above). *Structure of the lipase inhibitor BAY N 4605.* Molecular weight = 387; $K_i \sim 2X10^{-6}$M

Fig. 9 (right). *Effect of 1-30 mg BAY N 4605 in 2.1 g of olive oil/kg body weight on the postprandial serum triglyceride (TG) concentrations in rats*



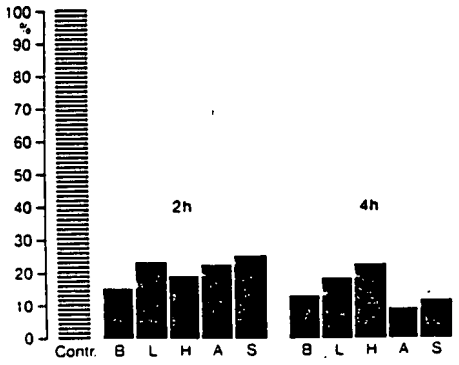


Fig. 10. *Effect of 30 mg BAY N 4605/kg on lipid radioactivity (^{14}C-triolein) in blood (B), liver (L), heart muscle (H), adipose tissue (A), and skin (S) (per cent of control) 2 and 4 h after oral administration of 2.1 g olive oil and ^{14}C-triolein/kg body weight.* Differences are significant ($P < 0.001$) vs control

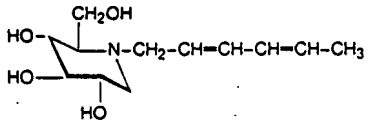


Fig. 11. *Structure of BAY N 2920.* Inhibitor of carbohydrate and lipid absorption

weight gain of rats which received standard chow. However, when a diet containing 14 g per cent fat instead of 4.2 g per cent fat in standard chow was fed, the body weight gain of the rats was significantly reduced.

Inhibitor of carbohydrate and lipid absorption

It was recently found that the deoxynojirimycin derivative BAY N 2920 (1, 5-dideoxy-1,5 [(hexa-2(E),4(E)-dienyl)imino]-*D*-glucitol)[23] (Fig. 11) did not only inhibit glucosidases, *in vitro* and *in vivo*, but also reduced the postprandial serum triglyceride increment in fat loading tests on rats (Fig. 12). In oral carbohydrate loading tests using sucrose or cooked

Loading test with:	ED_{50}, mg/kg body weight
Sucrose	0.2 mg BAY n 2920
Cooked starch	0.3 mg BAY n 2920
Olive oil	7.0 mg BAY n 2920
Mixed meal (ol. oil, CHO, protein)	1.0 mg BAY n 2920

Fig. 12. *ED_{50} values of BAY N 2920 for reduction of postprandial blood glucose increment (CHO loading) and serum triglyceride increment (fat or mixed meal loading) in rats*

starch the ED_{50} was 0.2 and 0.3 mg BAY N 2920/kg body weight, respectively. In oral fat loading tests (2.1 g olive oil/kg rat) the ED_{50} was much higher. Administration of 7 mg BAY N 2920/kg body weight reduced the postprandial serum triglyceride increment by 50 per cent in comparison to control rats. In loading tests with olive oil, carbohydrates and casein the effect of this agent was stronger. The ED_{50} was calculated to be 1 mg BAY N 2920/kg body weight. As with the deoxynojirimycin derivative BAY M 1099 this compound was almost completely absorbed from the intestine of rats and recovered from the urine. In toxicity experiments oral administration of 2000 mg BAY N 2920/kg body weight was well tolerated by mice and rats.

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Antiobesity activity of pluronic L-101

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Summary

Pluronic L-101, a hydrophobic surface-active agent, was a potent *in-vitro* inhibitor of human pancreatic lipase. When administered as a 1 percent or 3 percent dietary admix to meal-fed rats, pluronic L-101 produced a significant and dose-dependent decrease in body-weight gain while not affecting food consumption. Excretion of dietary fat in the feces was enhanced significantly in a dose-dependent manner during pluronic L-101 treatment. Pluronic F-68, a hydrophilic surface-active agent which was a poor *in-vitro* inhibitor of human pancreatic lipase, did not produce decreases in body-weight gain or increase fecal-fat excretion in rats. The decreased body-weight gain produced by pluronic L-101 was reflected in a decreased percentage of carcass fat; the percentage of carcass protein was unchanged. Liver wet weight was reduced significantly only at the 3 percent pluronic L-101 level. Serum levels of cholesterol, triglyceride and glucose remained unchanged. During pluronic L-101 treatment no overt signs of toxicity were observed.

Introduction

Agents which decrease the absorption of dietary lipids have been considered as an approach for therapy of obesity[3]. In recent years several classes of compounds have been examined with this in mind. Perfluorooctyl bromide, an intestinal coating agent[12], the antibiotic neomycin[9], non-absorbable dietary fats[1,14,15] surface active agents[2,10,13] and bile-salt sequestering agents such as cholestyramine[20] are a few that have been described in detail. Nonabsorbable dietary-fat replacements and certain surface-active agents are attractive as antiobesity agents since they might be expected to interrupt the function of pancreatic lipase, the enzyme responsible for the hydrolysis of emulsified dietary triglyceride.

Inhibition of pancreatic lipase can be accomplished by either direct active-site inhibitors or through the interruption of the bile-salt micelle complex. Nontoxic active-site inhibitors of pancreatic lipase have not been reported. However, *in-vitro* interruption of bile-salt micelles resulting in decreased pancreatic

lipase activity has been achieved[13,16,17]. Currently, two bile-salt sequestering hypocholesterolemic agents, colestipol and cholestyramine, have this property as an *in-vivo* side-effect[11,19]. Other agents more potent than these bile-salt sequestering agents have been sought, but signs of toxicity have limited their development.

The present study was conducted using pluronics L-101 and F-68, two non-toxic surface-active agents having markedly different physical properties. Pluronic L-101 was found to be a potent inhibitor of human pancreatic lipase and its antiobesity potential was evaluated *in vivo* in rats.

Experimental procedures

Animals and dietary treatment

Female rats (Charles River CD strain, 180-200 g) were housed individually in wire-bottomed cages in a temperature (22 °C) and light-regulated [12 h light (06.00 – 18.00 h) and 12 h dark] room. Rats had free access to a commercial diet (Purina Rodent Chow, Ralston Purina Co., St. Louis, Mo) and water.

Two weeks prior to experimentation rats were fasted 24 h then trained to consume within 2 h a high-fat control (10 percent corn oil) meal. Each rat received a food cup each morning at 09.00 h. The food cup was removed at 11.00 h. Within ten days rats were able to consume sufficient amounts of this diet (approximately 12 g/day) within the allotted time to gain weight near the normal rate.

The control 10 percent corn-oil meal consisted of 60 percent glucose, 20 percent vitamin-free casein, 10 percent corn oil (Mazola), 5 percent salt mixture, 1 percent vitamin mixture, and 4 percent cellulose. Experimental diets were prepared with the same caloric density (4.1 kcal/g) as the control 10 percent corn-oil diet. Pluronic L-101 (1 percent or 3 percent) or pluronic F-68 (3 percent) was added at the expense of the cellulose component.

Rats (n = 10 per group) were fed their respective control or experimental diets for 2 h daily (09.00 – 11.00 h) for 42 days. Food spillages were recorded and food consumptions were calculated daily. Body weights were recorded twice weekly.

Analytical methods

Fecal-fat elimination and absorption of dietary fat was determined by gravimetric analysis[4]. Feces were collected for three consecutive days during three periods of the study (Period 1 = day 7 to day 9; period 2 = day 20 to day 22; period 3 = day 38 to day 40). Feces were saponified in alchoholic-KOH, then extracted with hexane to obtain the free fatty acids.

On the final day of the study rats were decapitated and blood collected. Serum was prepared by centrifugation after the blood samples clotted on ice for 1/2 h. Liver and adipose tissues were removed, quickly placed on ice then stored at -20 °C. Enzymatic methods were used for the determinations of serum triglycerides, cholesterol and glucose[5]. Liver and adipose tissues were analyzed for lipids as described previously[5].

The eviscerated carcasses of the control and 3 percent pluronic L-101-treated rats were saponified in alcoholic-KOH for the determination of carcass lipid[22]. Carcass protein was determined by Kjeldahl nitrogen[18].

Inhibition of pancreatic lipase (EC 3.1.1.3) by pluronics L-101 and F-68 was determined by a titrimetric method described previously using human pancreatic lipase[5]. Inhibition constants for pluronics L-101 and F-68 were determined by the graphical method of Dixon & Webb[8].

All experiments were performed at least twice. Data were processed for outliers[7] and analyzed for significance using a two-tailed Student's *t*-test[21].

Materials

The cholesterol and glucose-test kits were from Abbott Labs., Pasadena Ca, and the triglyceride-test kit from CalBiochem., La Jolla, California Pluronics. L-101 and F-68 were kindly provided by BASF Wyandotte, Wyandotte, Michigan. Corn oil (Mazola) was purchased locally. All other chemicals were purchased from Sigma Chemical Co., St. Louis, Missouri and were of the highest quality available.

Results

As shown in Fig. 1a pluronic L-101 was a potent *in-vitro* inhibitor of human pancreatic lipase, demonstrating an inhibition constant (K_i) of 5.2 μM (0.02 mg/ml). In contrast, pluronic F-68 was a markedly less potent inhibitor exhibiting a K_i of 297 μM (2.5 mg/ml), a value 60-fold higher than pluronic L-101 (Fig. 1b).

The inhibition of pancreatic lipase by pluronics L-101 and F-68 was tested *in vivo* in rats by administering the compounds as either 1 percent or 3 percent dietary admixes for 42 days. Fecal-fat elimination, a measure of fat absorption, was determined during three periods of the experiment (Table 1). A dose response increase in fecal-fat elimination was observed for the 1 percent and 3 percent levels of pluronic L-101. The 1 percent pluronic L-101 dietary admix equal to 420 mg/kg body weight per day resulted in a significant increase over control of greater than 50 mg per day (175 percent of control) during the three periods examined. At the 3 percent pluronic L-101 level (1272 mg/kg body weight per day) fecal-fat excretion was increased by 125 mg per day over control, a 275 percent increase. The dose response of 1 percent and 3 percent pluronic L-101 was reflected also in decreased dietary-fat absorption (Table 1). Rats treated with 1 percent and 3 percent pluronic L-101 exhibited significant reductions in fat absorption of 3.4 percent for the 1 percent pluronic L-101 level and 9.8 percent for the 3 percent pluronic L-101 level. Pluronic F-68 produced no significant increases in fecal-fat elimination and, therefore, no changes in dietary-fat absorption (Table 1).

As can be seen from Fig. 2, rats fed diets containing pluronic L-101 or pluronic F-68 consumed similar amounts of food during the entire course of the study. There were no significant differences in food consumption at any time-point.

Rats which were treated with pluronic L-101 gained significantly less weight during the 42-day study (Table 2). However, there were no significant differences in food consumption – nor, hence in calories consumed – among the four groups of rats (Table 2).

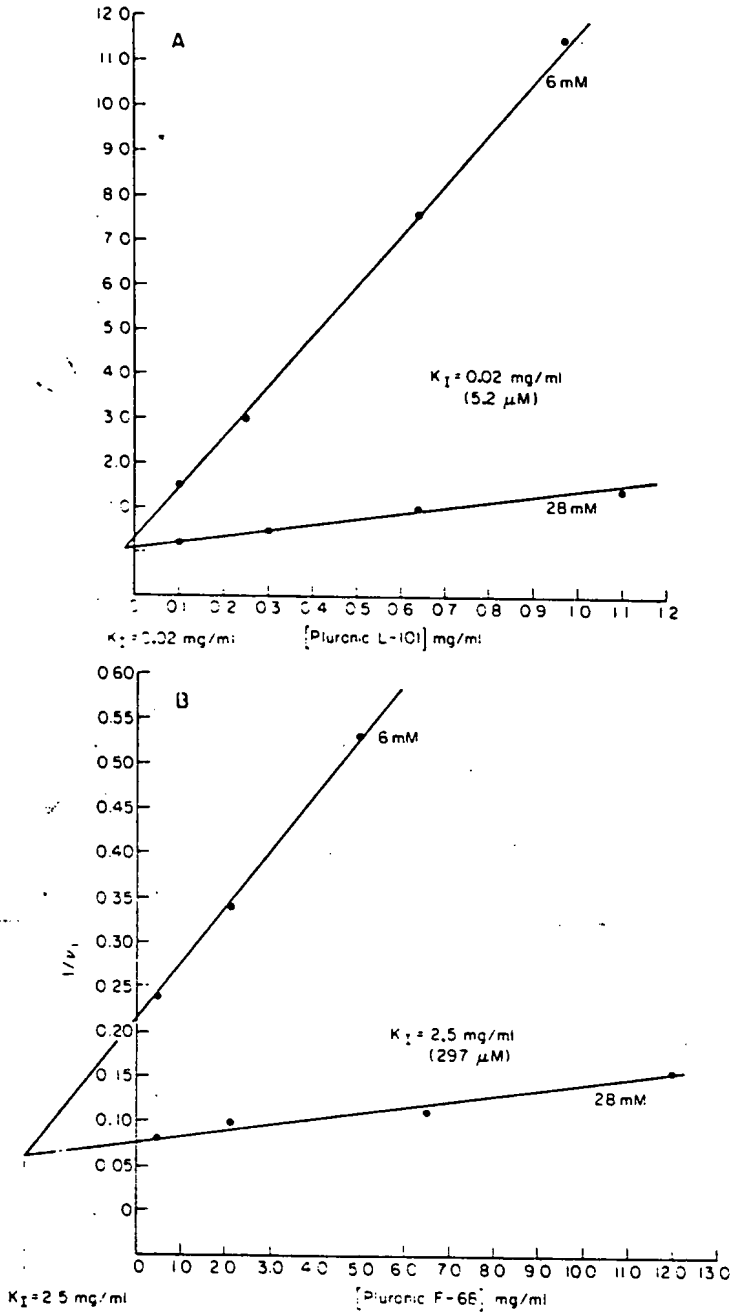

Fig. 1. Dixon plots of Pluronic L-101 (a) and F-68 inhibition of human pancreatic lipase (b). The indicated amount of inhibitor in 70 percent ethanol was added (max. vol. 200 μl) to the substrate emulsion (3.0 ml of either 6 mM or 29 mM triolein) and mixed thoroughly. Partially-purified human pancreatic lipase (10 μg protein) was added to initiate the reaction. The free fatty acids liberated (v_i = μmoles/min) were continuously titrated using a recording pH stat. Each point is the average of duplicate assays.

Table 1. Effect of pluronics L-101 and F-68 on dietary-fat elimination and fatty-acid absorption during a 42-day study[a]

Treatment	Dose[b]	Fat elimination[c]			Dietary-fat absorption
	mg/kg	period 1	period 2	period 3	%
			mg/day		
Control	—	80±2	73±9	65±7	95.1±0.4
Pluronic L-101	420[d]	123±4*	128±4*	125±8*	91.7±0.6*
Pluronic L-101	1272[e]	191±7*	221±7*	183±16*	85.3±1.2*
Pluronic F-68	1320[e]	74±4	91±4	59±4	95.7±0.2

[a]Rats were meal-fed daily from 09.00–11.00 h; ten rats per treatment group. Results are the average ± s.e. [b]Dose in mg per kg body weight per day, calculated from food consumption [c]Period 1 = day 7 to day 9; period 2 = day 20 to day 22; period 3 = day 38 to day 40 [d]Administered as a 1 percent dietary admix [e]Administered as a 3 percent dietary admix
*Significantly different from control $P < 0.05$.

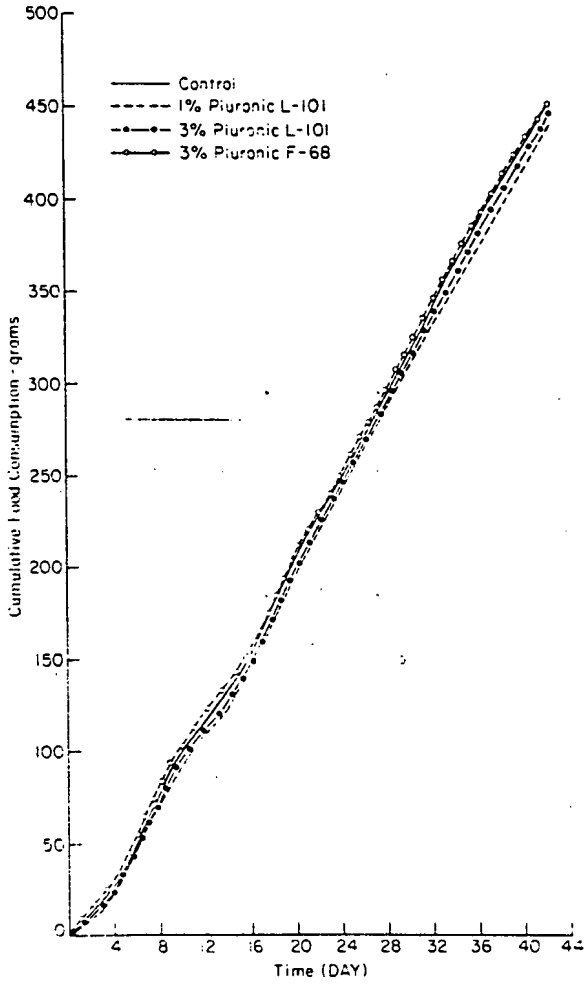

Fig. 2. Cumulative food consumption of rats fed pluronic L-101 and pluronic F-68. Groups of ten rats each were meal-fed for 2 h daily their respective diets for 42 days; control diet (solid line), 1 percent pluronic L-101 (dashed line), 3 percent pluronic L-101 (solid circles) and 3 percent pluronic F-68 (open circles). Food consumptions and spillages were determined daily

Table 2. Effect of pluronics L-101 and F-68 on body-weight gain and food consumption during a 42-day study[a]

Treatment	Dose[b] mg/kg	Body weight gain g	Food consumption[c] g/day	Efficiency[d] g gained/g consumed
Control	—	21±4	11.0±0.3	0.045±0.008
Pluronic L-101	420[e]	8±2*	10.5±0.3	0.017±0.010*
Pluronic L-101	1272[f]	2±4*	10.6±0.6	0.003±0.009*
Pluronic F-68	1320[f]	18±3	11.0±0.3	0.039±0.006

[a]Rats were meal-fed for 2 h daily (09.00 – 11.00 h). There were ten rats per treatment group. Results are the average ± s.e. [b]Dose in mg per kg body weight per day [c]Average per day for the entire study [d]Efficiency of food utilization = g body weight gained per g food consumed [e]Administered as a 1 percent dietary admix [f]Administered as a 3 percent dietary admix

*Significantly different from control $P < 0.05$

The significant decreases in body-weight gain for the rats treated with pluronic L-101 without changes in caloric intake were reflected in significantly reduced efficiencies of food utilization (g body weight gained/g food consumed) which are shown in Table 2. At the 1 percent and 3 percent pluronic L-101 levels the efficiency of food utilization was 0.017 ± 0.010 and 0.003 ± 0.009 respectively, values significantly lower than the control value of 0.045 ± 0.008. Rats treated with pluronic F-68 did not exhibit a significant decrease in the efficiency of food utilization (Table 2).

Liver wet weight and fatty-acid content of liver and adipose tissue from rats treated with either pluronic L-101 or pluronic F-68 showed only slight variations from control values (Table 3). Liver wet weight was reduced significantly only in rats treated at the 3 percent level of pluronic L-101. Liver fatty acids were reduced slightly but significantly at the 1 percent pluronic L-101 level when compared with control values. Pluronic F-68 did not have an effect on liver wet weight; however, liver fatty acids were reduced significantly to 87 percent of the control value. Adipose-tissue fatty-acid content was not significantly altered by treatment with either pluronic L-101 or pluronic F-68.

Analyses of rat serum for triglyceride, cholesterol and glucose content revealed no significant differences between the control-group values and any of the treatment groups (Table 4).

Carcass compositions from control and treated rats were performed at the termination of the study. Composition analyses were not performed at the initiation of the study in a separate group of animals.

Analyses of carcasses from control rats and rats treated at the 3 percent level of pluronic L-101 revealed an average significant decrease of 4 g of carcass fat (Table 5). The decrease of 4 g of carcass fat translated into a decrease in the percentage of fat in the carcasses (7.6 ± 0.6 percent) when compared to the control value (9.0 ± 0.8 percent). Although a 3.5 g difference in carcass protein was observed between control rats and rats treated with 3 percent pluronic L-101, when the protein was expressed as a percentage of carcass weight no difference from control was observed.

Table 3. Effect of pluronics L-101 and F-68 on liver in a 42-day study[a]

Treatment	Dose[b] mg/kg	Liver wet weight g	Liver fatty acids mg/g
Control	—	8.9±0.2	30±1
Pluronic L-101	420[c]	8.4±0.2	27±1*
Pluronic L-101	1272[d]	7.8±0.2*	29±2
Pluronic F-68	1320[d]	8.9±0.3	26±1*

[a]Rats were meal-fed 2 h daily (09.00 – 11.00 h). There were ten rats per treatment group. Results are the average ± s.e. [b]Dose in mg per kg body weight per day [c]Administered as a 1 percent dietary admix [d]Administered as a 3 percent dietary admix
*Significantly different from control, $P < 0.05$.

Table 4. Effect of pluronics L-101 and F-68 on serum parameters in a 42-day study[a]

Treatment	Dose[b] mg/kg	Triglycerides mg/100 ml	Cholesterol mg/100 ml	Glucose mg/100 ml
Control	—	85±7	105±4	164±7
Pluronic L-101	420[c]	89±10	109±5	164±5
Pluronic L-101	1272[d]	89±8	95±5	165±4
Pluronic F-68	1320[d]	92±7	105±8	169±5

[a]Rats were meal-fed 2 h (09.00 – 11.00 h). There were ten rats per treatment group. Results are the average ± s.e. [b]Dose in mg per kg body weight per day. [c]Administered as a 1 percent dietary admix. [d]Administered as a 3 percent dietary admix.
*Significantly different from control $P < 0.05$.

Table 5. Effect of pluronic L-101 on carcass fat and protein content in a 42-day study[a]

Treatment	Dose[b] mg/kg	Carcass fat[c] g	Carcass fat[c] % carcass weight	Carcass protein[d] g	Carcass protein[d] % carcass weight
Control	—	18.1±1.8	9.0±0.8	46.0±2.4	23.6±1.1
Pluronic L-101	1272	14.0±0.9*	7.6±0.6	42.5±2.2	23.5±0.8

[a]Rats were meal-fed 2 h daily (09.00 – 11.00 h). Ten rats per group. Results are expressed as the average ± s.e. [b]Dose in mg per kg body weight. Pluronic L-101 administered as a 3 percent dietary admix [c]Determined from saponified rat carcasses [d]Determined by Kjeldahl nitrogen
*Significantly different from control, $P < 0.05$.

Discussion

Pluronic polyols (polyoxyethylene-polyoxypropylene copolymers) are nonionic surfactants containing various percentages of the hydrophilic (polyoxyethylene) and hydrophobic (polyoxypropylene) components whose physical properties vary greatly depending upon the ratio of the hydrophilic and hydrophobic units. The two pluronics chosen for this study had opposite composition: pluronic L-101 was 90 percent hydrophobic and 10 percent hydrophilic while pluronic F-68 was 20 percent hydrophobic and 80 percent hydrophilic.

Hypolipidemic properties of pluronics have been reported previously by Bochenek & Rodgers[2]. In that report, pluronics similar to pluronic L-101 were reported to decrease the absorption of both triglyceride and cholesterol in rats administered by gavage an emulsion of pluronic surfactant containing cholesterol and triglyceride. Previous work from our laboratory has shown that pluronic L-101 decreased dietary-triglyceride absorption without effect on cholesterol absorption in rats trained to consume a synthetic high-fat meal[6]. From this work it was proposed that pluronic L-101 decreased the activity of pancreatic lipase thereby making dietary triglyceride unavailable for absorption. The present report confirmed the *in-vitro* inhibition of pancreatic lipase by pluronic L-101 (Fig. 1a). The inhibition constant (K_i) of 0.02 mg/ml (5.2 μM) for pluronic L-101 makes it the most potent, nontoxic inhibitor reported. The inhibition of pancreatic lipase activity may be due to an effect of the hydrophobic surfactant on the bile salt-enzyme-coenzyme complex. This was supported by the observation that pluronic F-68, a hydrophilic (water soluble) surfactant, was a very weak inhibitor of pancreatic lipase (K_i = 297 μM).

The translation of decrease *in-vitro* lipase activity to an *in-vivo* system was demonstrated by increased fecal-fat elimination and reduced body-weight gain in rats fed diets containing either 1 percent or 3 percent pluronic L-101 (Table 2). Pluronic F-68, a poor inhibitor of pancreatic lipase, did not enhance fecal-fat elimination in rats when administered as a 3 percent dietary admixture, nor did it significantly alter body-weight gain.

The decreases in body-weight gain of rats treated with the 1 percent and 3 percent levels of pluronic L-101 appeared to be due mainly to enhanced fecal-fat elimination and, to a lesser degree, to the slightly-decreased food consumption. The food consumption of rats in each treatment group was not significantly different from control. However, at the 1 percent pluronic L-101 level rats consumed 0.5 g per day less than controls resulting in a decrease of 86 kcal (359 kJ) over the 42-day study. Rats fed the 3 percent level of pluronic L-101 consumed an average of 0.4 g of diet less per day than controls resulting in a decrease of 68 kcal (284 kJ) during the course of the study. Using the efficiency of food utilization calculated for the control group (0.045 g gained/g diet consumed or 0.011 g gained/kcal consumed) rats treated at the 1 percent and 3 percent levels of pluronic L-101 would have decreased their body weight gain by only 1 g and 0.75 g, respectively.

The enhancement of fecal-fat elimination by pluronic L-101 was dose-dependent and averaged about 50 mg per day over control at the 1 percent level and 150 mg per day over control at the 3 percent level. This resulted in decreased dietary fat absorption of 3.4 percent and 9.8 percent for the 1 percent and

3 percent levels, respectively (Table 1).

From the decreases in body-weight gain not accounted for by the slight decreases in food consumption, it was calculated that in the rat a 3.4 percent decrease in daily fat absorption translated into a decrease in body-weight gain of about 0.3 g per day and a 9.8 percent decrease in daily fat absorption, observed at the 3 percent pluronic L-101 level, would result in a decrease in body-weight gain of almost 0.5 g per day. Although these decreases were not measurable on a daily basis, the differences from control became apparent within a week of treatment. It is noteworthy that although the rats were excreting increased levels of dietary fat and, therefore, experiencing an increased energy deficit they did not compensate for this deficit by increasing food consumption. This latter type of response has been observed in rats when their diets were diluted with nonabsorbable fiber or fat [4, 23].

Analyses of the carcasses of control rats and rats treated with pluronic L-101 for fat and protein content revealed a significant decrease in grams of body fat and a decrease in the percentage of the carcass fat (Table 5). Although the protein analysis revealed a decrease in total grams of carcass protein, when expressed as a percentage of the carcass no decrease was observed. These results indicate that the decreased fat absorption resulted in decreased carcass-fat deposition without effects on carcass protein.

Pluronic L-101 and F-68 produced no toxic signs in rats during the 42-day study. Hepatomegaly was not observed in any treatment group. Liver weight was reduced significantly at the 3 percent pluronic L-101 level (Table 3). Liver and adipose-tissue lipids were not changed to a biologically-significant degree. Furthermore, there were no changes from control in the serum values of cholesterol, triglycerides or glucose (Table 4). The lack of a hypocholesteremic effect of pluronic L-101 in the present study can be explained by the fact that in a previous study where hypocholesteremic activity was observed, the experimental rats were made hypercholesteremic by high levels of dietary bile salts and cholesterol[2].

The present study demonstrates that, by decreasing fat absorption through interference of pancreatic lipase, the problem of compensatory hyperphagia which is observed in rats during the administration of calorically-diluted diets containing nonabsorbable fiber or fat is avoided.

It also indicates that agents which decrease the absorption of dietary triglyceride may have use in weight-management programs in the treatment of obesity, and that such agents are worthy of further consideration and development. The concerns of this type of approach in the control of human obesity center around the effects of these agents on the absorption of nutrients other than fat, such as proteins and far soluble vitamins, and the possibility of negative long-term effects on the mucosal cells of the intestine. Answers to these questions await further animal experimentation.

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